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(19)(CA) **APPLICATION FOR CANADIAN PATENT** (12)

(54) Potential Target for an Immunotherapeutic Approach to Stop Huntington`s Chorea in Humans: cDNA and Protein Structures for an Intracellular Activator of the Human Glutamate Receptor, Brain Lactate Dehydrogenase and Vacuolar ATPase Reconstructed from the Antisense Strand of Baboon Urate Oxidase Gene

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Notice: This application is as filed and may therefore contain an incomplete specification.

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ABSTRACT

Recently it has been shown that very high concentrations of lactic acid accumulate in the brains of people with Huntington's disease and that the neurons which die in the brains of these patients are virtually overexcited to death by the neurotransmitter glutamate. There is no known cure for Huntington's chorea. A 216 base pair cDNA reconstructed from the antisense strand of baboon urate oxidase gene encodes a 64 amino acid, 7.6 kD, peripheral membrane associated protein which appears to be an intracellular activator of the glutamate receptor, brain lactate dehydrogenase and vacuolar ATPase. These three proteins acting together are capable of leading to the death of neurons and to deposits of high concentration of lactic acid observed in the brains of Huntington's disease victims. Although not yet identified, a similar protein is expected to be present in humans. Because the 7.6 kD protein is encoded by DNA homologous to the antisense strand of urate oxidase mRNA it cannot be expressed in the presence of a normal wild type urate oxidase gene, but mutations and/or deletions occurring in strategic regions of the gene can allow expression of the protein. Since this protein cannot be expressed in healthy humans, immunotherapeutic reagents directed against it should be useful for stopping Huntington's disease in humans without having any effect on other metabolic reactions.

SPECIFICATIONS

Huntington's chorea is a progressive and fatat neurological disease which starts in humans around age 40. There is no cure for the disease at present and until recently nothing was known about the biochemical basis of the disease. Recent results have led to the conclusion that faulty metabolism in brain cell mitochondria is involved, that high concentration of lactic acid occurs in the brains of Huntington's patients and that neurons in the brain are virtually excited to death by the neurotransmitter glutamate.

This invention relates to the discovery and reconstruction of a 216 base pair cDNA which is 100% homologous to nucleotides 197 - 403 on the antisense strand of the protein coding region of baboon urate oxidase gene. The cDNA shown in the embodiment of figure 1A encodes a 66 amino acid, 7.6 kD, protein shown in the embodiment of figure 1B. The domains in the protein which are overlined in figure 1B are putative intracellular activator regions for the following proteins: the glutamate receptor (glu), brain lactate dehydrogenase (ldh) and vacuolar ATPase (vase).

The cDNA is constructed by oligonucleotide synthesis on the ABI DNA synthesizer using the column method. Each DNA strand is constructed in 3 sections. The sections are purified by reversed phase HPLC and linked together in the appropriate manner. The full length chains are mixed in equimolar amounts and the cDNA is gel purified and processed in the express^{tm} system (Invitrogen corporation pTrcHis Xpress-Prokaryotic Expression and Purification system) according to recommendations of the manufacturer. The purified protein is used to prepare homologous antisera.

The glutamate receptor on brain neurons is over stimulated by glutamate, a highly active lactate dehydrogenase in brain neuron mitochrondia produces lactate from pyruvate and a vacuolar ATPase pumps protons accross the membraned into synaptic vesicles to maintain the supply of neurotransmitter; some of these protons combine with lactate to form lactic acid.

Because the mRNA encoding the 7.6 kd protein is antisense to the urate oxidase mRNA it cannot be expressed in the presence of a normal urate oxidase gene, but it can be expressed if the urate oxidase message contains point mutations or deletions in regions that are crucial for maintaining antisense repression.
Because the antisense protein is never expressed in normal cells, it is an ideal target for simple immunoterapeutic reagents with little possibility of producing adverse physiological reactions in the patient.

Normal urate oxidase protein is not expressed in humans because of two point mutations in the protein coding region of human urate oxidase gene; however, nucleotide sequences in the human gene is > 98 % homologous to the baboon gene and presumably could produce a non-functional mRNA that cannot be translated into urate oxidase. We believe the latter pseudo mRNA can function, in humans, as antisense repressor to a protein homologue of the 7.6 kD baboon protein.

Based on the mechanisms described above, we have concluded that immunotherapeutic reagents directed against the 7.6 kD protein should stop over activation of glutamate receptors and production of lactic acid in the brains of people carrying a mutation in a human gene homologous to the 7.6 kD, antisense, baboon gene. Stoping overstimulation by glutamate and the action of the overactive lactic dehydrogenase should prevent the pathophysiological symptoms of Huntington's disease in humans.

The Embodiments of the discovery in which an exclusive property or privilege is claimed are defined as follows:

1. The cDNA and protein described in the embodiment of figures 1A and 1B.

2. The cDNA and protein resembling or equivalent to those described in the embodiment of figure 1A and 1B isolated from humans.

3. Any diagnostic and or therapeutic reagents produced from or against parts or the entirety of the cDNAs described in clause 1 and 2 above or from any experimentally or naturally mutated form of these cDNAs.

4. Any diagnostic and or therapeutic reagents including monoclonal antibodies, polyclonal antibodies, fusion proteins, antipeptide reagents etc., made from or against any or all the amino acid sequences described in the embodiment of figure 1B and in clause 2 above, or from any experimentally or naturally mutated form of these proteins.

FIGURE 1

(A)

5' AAA TGC ATG GAC ATG CTT AAC TCC ATT GTT TTC
AAG ACG CCT CCA AGG GAT TTC TTC CAC GTA GAC
TTG TGC TCG GAT TAC ATG GTT AAA AGA AGA AAG
AAA ATA CTC ACA AAT ATT CAC ACC AAA GGC TTC
TAT CCT TTT GAT TCC CTT AAT CTT TGC CAA CAC
ATG AAC TGT GTT CTT GAT GGT GTC TGC AGG GAT
GAT GAT ATC TGA ATTATCTCC

(B)